

# Physiological roles of animal succinate thiokinases

## Specific association of the guanine nucleotide-linked enzyme with haem biosynthesis

T.M. Jenkins and P.D.J. Weitzman

*Department of Biochemistry, University of Bath, Bath BA2 7AY, England*

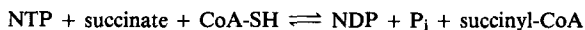
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The discovery of two distinct succinate thiokinases in mammalian tissues, one (G-STK) specific for GDP/GTP and the other (A-STK) for ADP/ATP, poses the question of their differential metabolic roles. Evidence has suggested that the A-STK functions in the citric acid cycle in the direction of succinyl-CoA breakdown (and ATP formation) whereas one role of the G-STK appears to be the re-cycling of succinate to succinyl-CoA (at the expense of GTP) for the purpose of ketone body activation. A third metabolic participation of succinyl-CoA is in haem biosynthesis. This communication shows that in chemically induced hepatic porphyria, when the demand for succinyl-CoA is increased, it is the level of G-STK only which is elevated, that of A-STK being unaffected. The results implicate G-STK in the provision of succinyl-CoA for haem biosynthesis, a conclusion which is further supported by the observation of a high G-STK/A-STK ratio in bone marrow.

Succinate thiokinase; Guanine nucleotide; Heme synthesis; Porphyrin synthesis; Porphyrin; Mitochondrial metabolism

### 1. INTRODUCTION

Mammalian succinate thiokinase (succinyl-CoA synthetase) (STK) catalyses the following reversible reaction:



where NTP and NDP represent nucleoside triphosphate and diphosphate. We have recently reported the existence and separation of two distinct STKs in mammalian tissues specific for guanine (G-STK) (EC 6.2.1.6) or adenine (A-STK) (EC 6.2.1.5) nucleotides [1] and have investigated the possible distinct metabolic roles for each enzyme [2,3]. These studies have suggested that, in vivo, A-STK and G-STK may be responsible for catalysing opposing directions of the STK reac-

tion. In mammalian brain G-STK shows a marked elevation in activity with increased ketone body utilization [2], thus supporting the proposal that the re-cycling of succinate to succinyl-CoA, required for ketone body activation, is achieved directly by the action of G-STK. On the other hand, in the eukaryotic parasitic protozoan *Trypanosoma brucei*, during its transformation from bloodstream to procyclic cell form, it is A-STK, not G-STK, which accompanies a concerted rise in the activity of other citric acid cycle enzymes, thus implicating A-STK as an integral component of the citric acid cycle operating in the direction of succinyl-CoA hydrolysis [3].

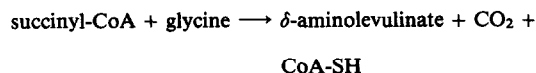
The other main metabolic role for succinyl-CoA, in the mammalian mitochondrion, apart from its involvement with the citric acid cycle and ketone body activation, is in haem biosynthesis. The first and rate-limiting enzyme of the haem biosynthetic pathway,  $\delta$ -aminolevulinic acid syn-

*Correspondence address:* P.D.J. Weitzman, Department of Biochemistry, University of Bath, Bath BA2 7AY, England

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thetase (ALA-S) (EC 2.3.1.37), utilises succinyl-CoA in the following reaction:



All mammalian cells contain haemoproteins and possess the capability to synthesize haem [4]. However, the highest rates of haem biosynthesis are located in bone marrow, responsible for erythropoiesis, and in the liver.

There are a number of disease states of haem biosynthesis, collectively known as porphyrias [4]. In hepatic porphyria, perturbation of haem biosynthesis occurs, due to a deficiency or inhibition of an intermediate enzyme. This leads to overproduction and excretion of porphyrin precursors due to derepression of ALA-S synthesis [5]. As a result, the biosynthetic pathway, normally under strict negative feedback control, then operates with elevated levels of ALA-S, thus presenting increased demand for succinyl-CoA. Such demand may be met by an enhanced activity of STK.

Here, we present evidence for the involvement of G-STK in haem biosynthesis. Hepatic porphyria was induced in mice by the drug 3,5-diethoxycarbonyl 1,4-dihydrocollidine (DDC) and the levels of A-STK and G-STK activities were measured. Additionally, bovine bone marrow (sternum) was investigated with respect to A-STK and G-STK activities in view of its essential role as a site for erythropoiesis.

## 2. EXPERIMENTAL

### 2.1. Preparation of bovine bone marrow extracts

Bovine bone marrow (sternum) was obtained immediately after slaughter. 10 g tissue (possessing a red ossified appearance) was added to 30 ml ice-cold 0.1 M Na/K phosphate buffer (pH 8.0), 1 mM EDTA and homogenized in a Sorvall Omni-mixer (4 × 15-s bursts interspersed with cooling, at full power). After centrifugation (30000 × g for 30 min at 4°C) the supernatant solution was used without further treatment.

### 2.2. Induction of hepatic porphyria in mice

Female mice (strain CFLP), each about 30 g and individually housed, were given drinking water ad libitum and starved for 24 h. Each mouse of the treatment group then received 5 g laboratory chow containing 10 mg DDC (kindly supplied by Dr F. De Matteis, MRC Toxicology Unit, Carshalton); the control group received only 5 g laboratory chow. Mice were killed 24 h later and livers were immediately removed. Aliquots (0.5 g) of control and treated livers were retained for total liver porphyrin

determination; the remainder of the livers were pooled into control and treated groups for the preparation of mitochondria.

### 2.3. Preparation of mouse liver mitochondrial extracts

Liver tissue, after gall bladder removal, was diced into ice-cold extraction buffer containing 0.25 M sucrose, 50 mM Tris-HCl buffer (pH 8.0), 1 mM EDTA, 0.1 mM EGTA and 0.1% albumin (fatty acid free). Homogenization (5 strokes) in a Potter-type homogenizer (0.3 mm gap) was followed by differential centrifugation, first to remove cell debris (1600 × g for 10 min at 4°C) and secondly to pellet mitochondria (30000 × g for 25 min at 4°C). Mitochondria were then washed in fresh extraction buffer containing no albumin. Finally, mitochondria were resuspended in 0.1 M Na/K phosphate buffer (pH 8.0) containing 0.05% (v/v) Triton X-100 and disrupted by ultrasonication (MSE 100 W sonicator operated at 40 W for 4 × 15 s with cooling). After centrifugation (30000 × g for 30 min at 4°C) the supernatant solution was used without further treatment.

### 2.4. Measurement of enzyme activities and protein concentrations

STK and citrate synthase were measured polarographically [6] as described in [7,8]. STK assays contained 1 mM ADP or GDP. Protein concentrations were determined as in [9].

### 2.5. Measurement of total liver porphyrins

Total liver porphyrins were extracted into 10.2 M perchloric acid/ethanol mixture (1:1) and measured fluorimetrically in an Aminco-Bowman spectrophotofluorimeter using an internal mesoporphyrin standard [10].

## 3. RESULTS AND DISCUSSION

Porphyrin mouse liver, gall bladder contents and prepared liver mitochondria were all characteristically dark red in appearance when compared with controls. The total liver porphyrin content of DDC-treated mice was increased 100-fold, from a control value of 4 nmol/g liver to a DDC-treatment value of 406 nmol/g liver, thus confirming the porphyrin state.

The initial and final steps of the haem biosynthetic pathway occur within the mitochondrion [11]. Ferrochelatase (EC 4.99.1.1), the final enzyme of the pathway, catalyses the insertion of ferrous iron into protoporphyrin IX [4]. In the liver of DDC-treated animals, transfer occurs of the 4-methyl group from DDC to one of the pyrrole nitrogens of protoporphyrin IX, converting it to *N*-methylprotoporphyrin [12] which is a potent irreversible inhibitor of ferrochelatase. The decrease in haem production removes the negative feedback control on the system and leads to overproduction of ALA-S and subsequently haem precursors [5].

Table 1

Effect of porphyria on citrate synthase and STK activities in mouse liver mitochondria

Treatment	Activity (nmol/min per mg protein)			Ratio G-STK/A-STK
	Citrate synthase	G-STK	A-STK	
Control liver mitochondria	8.9 ± 0.8	3.7 ± 0.4	1.5 ± 0.5	2.5
DDC-treated liver mitochondria	8.7 ± 0.5	7.4 ± 1.1	1.6 ± 0.3	4.6

This increase in ALA-S activity and demand for succinyl-CoA can be seen to be accompanied by an increase in G-STK activity (table 1). Both citrate synthase and A-STK showed no significant change in activities, whereas G-STK doubled in specific activity in the porphyric state compared with the control.

Significantly, bovine bone marrow (sternum) possesses the highest G-STK/A-STK ratio of any mammalian tissue so far examined. Thus, we found the specific activities of G-STK and A-STK to be 2.5 and 0.33 nmol/min per mg protein respectively, giving a ratio of 7.6. This value is considerably greater than the highest ratio reported for any other tissue, i.e. 3.5 for rat kidney [1]. This result, taken together with the sharp increase in mouse liver G-STK in the porphyric state, offers strong support to our proposal that G-STK does indeed supply succinyl-CoA for haem biosynthesis.

Over 20 years ago it was demonstrated that hepatic porphyria is accompanied by an elevation of STK activity [13]. However, at that time it was not known that liver and other mammalian tissues contain both A-STK and G-STK enzymes. Our recent discovery of these two enzymes has made it pertinent to investigate their differential response to the porphyric condition.

This communication, together with the report on G-STK involvement with ketone body activa-

tion [3], emphasises the distinct metabolic roles of A-STK and G-STK in mammalian cells. It would seem that in the mitochondrion the STK reaction is required to operate in both directions at the same time (citric acid cycle flux and porphyrin biosynthesis/ketone body activation). To achieve this bi-directionality, two STKs are required, possessing mutually exclusive nucleotide specificity and control.

In conclusion, we propose that A-STK is involved in energy conservation in the citric acid cycle, while G-STK is responsible for the supply of succinyl-CoA for other important metabolic needs. Further studies are currently aimed at exploring the fine level organisation and association of the two enzymes within the mitochondrion.

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